Marker identification and phylogenetic analysis of saline tolerant rice varieties

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Submission date: 06-Mar-2023 07:28AM (UTC+0700)
Submission ID: 2029572002
File name: 10_Artikel_IOP2.pdf (927.4K)
Word count: 2314
Character count: 12939

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To cite this article: Suprayogi *et al* 2021 *IOP Conf. Ser.: Earth Environ. Sci.* **746** 012014

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Abstract. Selection for saline tolerant rice genotypes has been based on morphological or physiological character. These two characters are phenotypically affected by environmental factors, such that, their reliabilities for selection criteria are obscure. Molecular marker assisted selection has been reported to be an effective and efficient selection method as molecular markers are not influenced by environmental factors. The objectives of this study were: (1) to describe the phylogenetic relationship of rice genotypes based on SSR markers, and (2) to know the reliability of microsatellite markers for phylogenetic analysis of rice genotypes in relation to salinity tolerance. The research was conducted from July to October 2020 at Plant Breeding and Biotechnology Laboratory, Faculty of Agriculture, Jenderal Soedirman University, Purwokerto, Indonesia. DNA extraction was carried out using CTAB method. Agarose gel electrophoresis was used for determination of genomic DNA quality and concentration. PCR was performed using 2720 Applied Biosystems Thermal Cycler. Seventeen microsatellite primers, namely: RM129, RM156, RM222, RM223, RM224, RM241, RM292, RM342B, RM444, RM426, RM493, RM519, RM528, RM1287, RM3412, RM8094, and Wn11463, were analyzed on eight rice genotypes, namely: Inpari Unsoed-79 Agritan, Cisadane, Atomita-2, Pelopor, Dendang, Lambur, Siak Raya, and IR 29. Extraction of genomic DNA has resulted in high quality DNA of 100 ng/μL concentration. Phylogenetic analysis demonstrated that the eight genotypes could be grouped into two clusters. Tolerant variety of Inpari Unsoed-79 Agritan was distantly apart from the susceptible variety IR29.

1. Introduction

Salinity has been a serious constraint for rice production across many coastal areas in Indonesia where rice-based farming systems predominate [1] [2], especially during the dry season and at the start of the wet season due to intrusion of sea water [3]. Despite rice is a salt-sensitive crop particularly during early vegetative and later at reproductive stages [1] [3] [4], it is one of the few crops that can thrive on salt-affected soils because of its ability to grow well in standing water. Consequently, rice has been recommended as volunteer crop for desalinization of salt affected lands [5] [6]. Therefore, development of saline tolerant varieties has been considered as the most reliable approach to increase rice production in saline prone coastal areas [4] [7].

Effective and efficient method of selection is a pivotal tool to assure the gaining of true genotype in breeding of saline tolerant rice varieties. Selection of salt tolerant rice genotypes has so far been based

on morphological or physiological characters. This conventional methods of plant selection for salt tolerance are not easy because of the large effects of the environment and low narrow sense heritability of salt tolerance [7] [8].

DNA markers has been reported to be the best candidates for efficient evaluation and selection of true genotype. This technology permits reduction of time and increase accuracy of crop breeding program where pronounced effects of environment lead to poor selection efficiency [7] [9]. Among the DNA markers, microsatellites or simple sequence repeats (SSRs) has been reported to be a reliable marker to find out salt tolerant rice genotypes. Three selected SSR markers, RM7075, RM336 and RM253 had been used to evaluate rice genotypes for salt tolerance [7]. For this, phenotypic and genotypic evaluation for salinity tolerance was done at the seedling stage. These markers showed polymorphism and were able to discriminate salt tolerant genotypes from susceptible. Meanwhile, an analysis of 100 SSR markers on 140 IR29/Pokkali recombinant inbreed lines (RILs) confirmed the location of the Saltol QTL between RM23 and RM140 on chromosome 1 and identified additional QTLs associated with tolerance. Saltol mainly acts to control shoot Na^+ /K^+ homeostasis [3]. On the other study, 28 SSR primers had been used to estimate genetic diversity among 30 rice genotypes [10].

The objectives of this study were (1) describe the phylogenetic relationship of rice genotypes based on SSR markers, and (2) to know the reliability of microsatellite markers for phylogenetic analysis of rice genotypes in relation to salinity tolerance.

2. Material and methods

2.1. Plant materials and DNA extraction

Eight rice genotypes, namely: Inpari Unsoed-79 Agritan, Cisadane, Atomita-2, Pelopor, Dendang, Lambur, Siak Raya, and IR 29 were used for DNA extraction material. Inpari Unsoed-79 Agritan is a saline tolerant rice variety produced by Faculty of Agriculture, Jenderal Soedirman University, and released in 2014 by the Ministry of Agriculture, Atomita-2 is also saline tolerant rice variety produced by National Nuclear Energy Agency of Indonesia (BATAN), IR 29 is a salt susceptible variety [3], Siak Raya and Dendang are swampy rice varieties, while Pelopor and Lambur are common commercial rice varieties. Three weeks old rice seedlings grown on petri-plates with H_2O were used as source of leaves genomic DNA.

DNA extraction was carried out using CTAB method [11]. Agarose gel electrophoresis was used for determination of genomic DNA quality and concentration, and confirmed using Implen Nano-Spectrophotometer.

2.2. DNA marker amplification

DNA marker amplification was performed using 2720 Applied Biosystems Thermal Cycler. Seventeen rice microsatellite primers, namely: RM129, RM156, RM222, RM223, RM224, RM241, RM292, RM342B, RM444, RM426, RM493, RM519, RM528, RM1287, RM3412, RM8094, and Wn11463, were analyzed on genomic DNA of Inpari Unsoed-79 Agritan, Cisadane, Atomita-2, Pelopor, Dendang, Lambur, Siak Raya, and IR 29. Polymerase Chain Reactions were performed in an individual 25 μL PCR tube each containing a reaction mixture of: 1 μL of 100 ng genomic DNA, 12.5 μL of PCR Master Mix (Thermo Fisher), 1 μL forward and reverse primers, and 9.5 μL nuclease free water.

The PCR cycle was initiated by an initial denaturation of 5 min at 95^{o}C, and followed by 35 cycles of 1 min for denaturation at 95^{o}C, 1 min annealing (temperature dependent on the individual primer), and 1 min extension at 72^{o}C followed by final extension of 5 min at 72^{o}C. Primer sequences and annealing temperature are presented in Table 1.

Table 1. Annealing temperatures of the primers used in this study.

Primer	Forward	Reverse	TA (°C)	Ref
RM426	ATGAGATGAGTTCAAGGCCC	AACTCTGTACCTCCATCGCC	58	[12]
RM129	TCTCTCCGGAGCCAAGGCGAGG	CGAGCCACGACGCGATGTACCC	58	[12]
RM342B	CCATCCTCCTACTTCAATGAAG	ACTATGCAGTGGTGTCACCC	58	[12]
RM444	GCTCCACCTGCTTAAGCATC	TGAAGACCATGTTCTGCAGG	58	[12]
RM241	GAGCCAAATAAGATCGCTGA	TGCAAGCAGCAGATTTAGTG	55	[12]
RM292	ACTGCTGTTGCGAAACGC	TGCAGCAAATCAAGCTGGAA	53,6	[13]
RM528	GGCATCCAATTTTACCCCTC	AAATGGAGCATGGAGGTCAC	55	[12]
RM222	CTTAAATGGGCCACATGCG	CAAAGCTTCCGGCCAAAAG	53,6	[13]
RM224	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCGGG	53,6	[13]
RM223	GAGTGAGCTTGGGCTGAAAC	GAAGGCAAGTCTTGGCACTG	55	[13]
RM1287	GTGAAGAAAGCATGGTAAATG	CTCAGCTTGCTTGTGGTTAG	55	[13]
RM519	AGAGAGCCCCTAAATTTCCG	AGGTACGCTCACCTGTGGAC	55	[13]
RM 8094	AAGTTTGTACACATCGTATACA	CGCGACCAGTACTACTACTA	54	[13]
RM493	TAGCTCCAACAGGATCGACC	GTACGTAAACGCGGAAGGTG	55	[14]
RM3412	AAAGCAGGTTTTCCTCCTCC	CCCATGTGCAATGTGTCTTC	55	[14]
RM156	GCCGCACCCTCACTCCCTCCTC	TCTTGCCGGAGCGCTTGAGGTG	67	[13]
Wn11463	TCCTCCTTCTCTCGCAAC	GATCCACTCGTCACAGG	54,9	[13]

Agarose gel in a horizontal electrophoretic system filled with 1x TBE. Ethidium bromide was used for intercalating agent of DNA fragment. Visualization of DNA was carried by submerging the Agarose gel in 1x TBE for 30 minutes, followed by gel-doc photography under UV light.

2.3. Phylogenetic analysis

Clearly resolved, unambiguous bands were scored visually for their size difference with each primer. The scores were obtained in the form of matrix with '1' and '0', which indicate the greater and smaller size of PCR product (DNA band) in each variety respectively. Phylogenetic analysis was carried out using NTSYS 2.2.

3. Results and discussions

Extraction of genomic DNA has resulted in high quality DNA (Figure 1). Genomic DNA Quantification using nano-spectrophotometer demonstrated that the average concentration ranged between 100 ng/μL to 250 ng/μL.

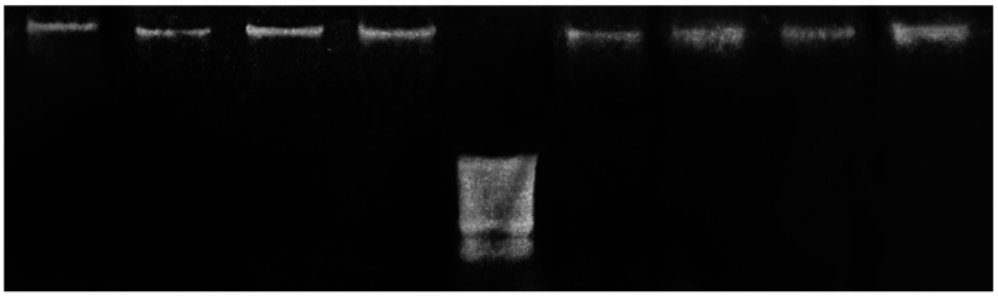

Figure 1. Genomic DNA resulted from CTAB DNA extraction method. From left to right: Inpari Unsoed-79 Agritan, Cisadane, Atomita-2, Pelopor, Hyperladder DNA 100 bp (Bioline), Dendang, Lambur, Siak Raya and IR 29.

All the 17 primers were able to produce polymorphic amplified products. Figure 2 showed PCR products of RM224, RM342B, RM519 and RM 1287 (Not all pictures of the PCR products are presented in this publication)

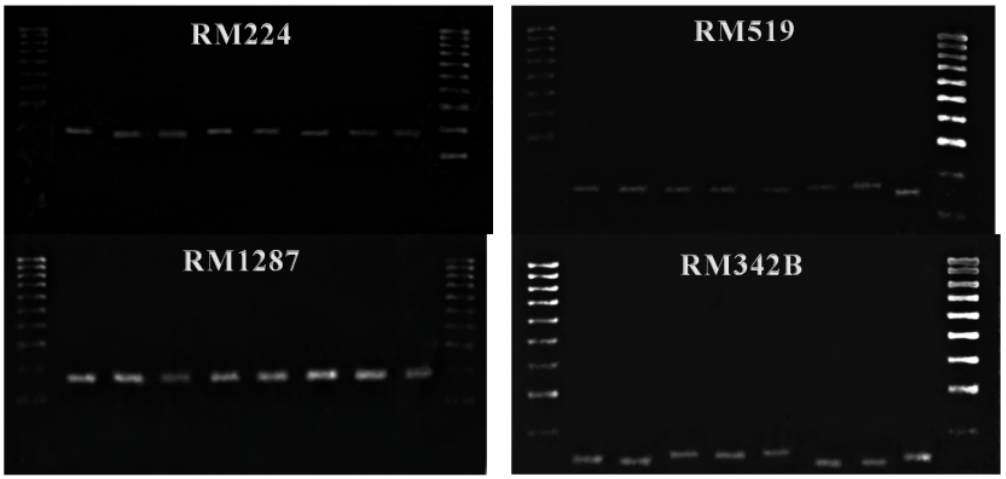

Figure 2. PCR products of RM224, RM519, RM 1287, RM342B and Hyperladder 100bp (Bioline). From left to right: Inpari Unsoed-79 Agritan, Cisadane, Atomita-2, Pelopor, Dendang, Lambur, Siak Raya and IR 29.

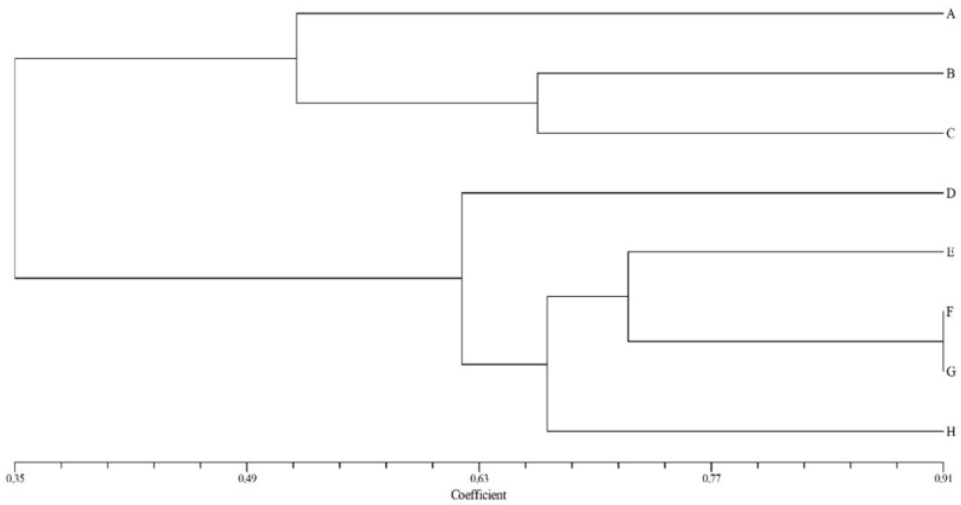

Figure 3. Phylogenetic tree of the eight rice genotypes. A= Inpari Unsoed Agritan-79, B= Cisadane, C= Atomita 2, D= Pelopor, E= Dendang, F= Lambur, G= Siak Raya, dan H= IR 29.

Phylogenetic analysis demonstrated that the eight genotypes could be grouped into two main clusters (Figure 3). It is interesting that based on 17 SSR markers, the tolerant variety Inpari Unsoed-79 Agritan was distantly apart from the susceptible variety IR29. Inpari Unsoed-79 Agritan is the progeny of the cross Cisadane (commercial cultivar) and Atomita-2 (saline tolerant rice variety [15]. In this phylogeny, Inpari Unsoed-79 Agritan, Cisadane and Atomita-2 are in the same group and closely related. Indicating that the SSR markers used in this phylogenetic study might be reliable for salt tolerance analysis. For instance, Wn11463 was reported to be significantly associated with salinity tolerance [16].

4. Conclusion

Phylogenetic analysis demonstrated that the eight genotypes could be grouped into two clusters. All the 17 SSR markers could be used to identify genetic relationship of rice genotypes in relation to salinity tolerance.

Acknowledgement

This research was funded by the Grant of Jenderal Soedirman University for Competence Improvement Research 2020. The authors thank to Iwan, University Research Lab technician, and Kartini, Plant Breeding and Biotechnology Laboratory technician.

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